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A sample-to-answer labdisc platform integrated novel membrane-resistance valves for detection of highly pathogenic avian influenza viruses

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ABSTRACT

This work described a novel labdisc platform adopting membrane-resistance (MembR) valves for fully automated sample-to-answer detection of highly pathogenic avian influenza viruses (HPAIVs). The MembR valves as the passive valves were successfully developed by utilizing different hydrophobic or hydrophilic polycarbonate membranes with superfine pore sizes and used in experiments. It allowed a large range of effective rotational speed for fluid control on the disc from 500 to 4750 rpm. In the platform, six MembR valves enabled effective pre-storage and manipulation of RNA extraction reagents under five different rotational speeds. With the help of MembR valves, all the processes for molecular diagnosis including sample lysis, RNA extraction and purification, and specific RNA detection using real-time reverse transcription loop-mediated isothermal amplification (RT-LAMP) were integrated on the single disc. We demonstrated that samples of three HPAIVs H7N3, H7N9, and H9N2 and two other influenza A subtypes H1N1 and H3N2 can be automatically analyzed on the centrifugal disc within 70 min. All experiments were applied in a 4 kg portable, laptop controlled point-of-care device. Furthermore, the platform provided a closed non-contact heating with accurate temperature control for POCT nucleic acid diagnosis.

1. Introduction

Highly pathogenic avian influenza viruses (HPAIVs) can cause respiratory or systemic infection in poultry and waterfowls to utterly devastate poultry husbandry and can spread in long-distance migrations of wild birds [1–4]. Since the viruses are also able to infect human beings with pandemic potential and cause death, they pose a grave threat to public health [5,6], as well as adversely impact on international trade and global economy [6–8]. Thus, detecting and monitoring the prevalence of HPAIVs as well as developing antiviral drugs and vaccines against HPAIVs have become great concerns to governments and health officials [9–11]. The establishment of rapid detection and diagnosis of HPAIVs can contribute to the early diagnosis and treatment of HPAIV-infected patients, meanwhile, it will facilitate epidemic surveillance and technological accumulation to provide early data and effective measures for coping with emergence of influenza pandemic [12,13]. Currently, nucleic acid analysis as an accepted method for accurately identification of influenza virus subtypes is mostly performed in central laboratories requiring a long turnaround time, which may increase the risk of disease transmission and greatly delay treatments and epidemic controls [12,14]. Therefore, there is still an urgent need for a diagnostic platform that can rapidly detect HPAIV infections at the point-of-care testing (POCT).

Most of existing commercial POCT approaches, such as test papers, are generally built up on simple principles with easy operation, so that they can only perform some preliminary tests [15,16]. Compared with these approaches, centrifugal microfluidics is regarded as one of the most promising technologies to realize advanced and complex processes on POCT systems for versatile and accurate analyses [17,18]. It can establish a process chain on a simple-structure disc-shaped microfluidic

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chip which combines an enormous breadth of available unit operations including liquid transport, valving, mixing, metering, aliquoting, etc. [19]. The whole process chain can be controlled solely by the rotational speeds of the centrifugal microfluidic chip [20,21], that improves the integration, miniaturization and automation of analysis workflows for different applications involving molecular diagnoses [22–25]. immunoassays [26–28], clinical chemistries [29,30], cell handling and analyses [31,32], and the analyses of water [33,34], food [35], and soil [36,37].

Recently presented centrifugal microfluidic devices are aiming at rapid analysis and easy to operate by non-professionals avoiding sample contaminations and operational pollutions [18,38]. These devices can implement the whole analysis by the integration of a disposable kernel centrifugal cartridge, a centrifuge, temperature controllers, optical readers, as well as other low-cost, mature techniques and modules in a light-weight device. Due to the advantage of strong practicability for field detections, many major diagnostic companies have already launched their centrifugal microfluidic-based products to the market [18]. In particular, the development of the centrifugal microfluidics clearly trends to the full integration of a complex sample-to-answer analysis in order to match requirements of ideal POCT detection [14,17,39]. For instance, the whole high-throughput genetic analysis process including nucleic acid extraction, amplifications for multiple targets, and parallel detections of amplicons has been demonstrated in a sample-in-answer-out manner on the centrifugal microfluidic chip. However, the centrifugal microfluidic-based solution is still at the initial stage that requires continuous accumulation of experience and technique innovation [18,22].

For nucleic acid diagnostics based on centrifugal microfluidics, prestorages of multiple reagent solutions and solution manipulations are two critical techniques to realize sample-to-answer analysis. Kim et al. introduced ferrowax valves and a laser diode in their integrated centrifugal microfluidic device enabling the pre-storages of buffers and automatic detection of foodborne pathogen with the help of programmed valve control [40]. Obviously, the accessories like the ferrowax, laser diode, test strips etc., would probably increase the complexity of system, hence reducing its reliability for the POCT application [18]. Roy et al. fabricated a series of capillary valves with superfine inside dimensions for the sequential control of liquid flow on a lab-on-disc chip [41]. The chip combined cell lysis, polymerase chain reaction (PCR), amplicon digestion and microarray diagnosis, but its process chain was time-consuming. Jung et al. and Oh et al. presented ring capillary valves in their systems for H1N1 virus analysis and foodborne pathogen detection, respectively, which is an ingenious design to integrate solution reservoir and liquid control together [39,42]. However, both systems manipulated only three different solutions for RNA/DNA purification, loop-mediated isothermal amplification (LAMP), and real-time detection step by step, the absence of virus/bacteria lysis made their capability for sample-to-answer analysis unsatisfactory. Czilwik et al. developed an excellent LabDisk system adopting magnetic bead manipulation instead of solution manipulation [43]. The auxiliary magnets were utilized for the control of bead motion in the network of channels enabling DNA extraction prior to real-time PCR procedures. Stumpf et al. further improved the LabDisk system by using stick-packs for prestorage of reagents and employed real-time reverse transcription PCR for H3N2 virus detection [14]. Their system provided striking demonstrations for the high-performance of molecular analysis on centrifugal microfluidic platforms except that it required a prolonged analysis time. Loo et al. introduced microball valves in their integrated lab-on-disc for sample-to-answer detection of bacterial infection [44]. Whereas the platform is suitable for the detection in the laboratory rather than POCT analysis, because the micro-ball is likely to be out of the close position during transportation that makes the valve invalid. In addition, none of the above-mentioned platforms except that presented by Czilwik and Stumpf, provided a closed heating container for the accurate temperature control to its centrifugal chip. Apparently, the stability of detection reactions that supported by an open-type heating component is susceptible to ambient temperature, especially in cold conditions. Because, inaccurate and unstable reaction temperature directly impacts on the combining capacity of polymerase to primers with different base sequences [45], even Bst polymerase in LAMP reaction possesses a relatively wide range of activation temperatures (60–65 °C). Thus, most of those platforms may not be perfect for POCT applications.

Herein, we present a novel membrane-resistance (MembR) valve for effective prestorage and manipulation of solution. Based on that, MembR valve-integrated centrifugal microfluidic platform for HPAIVs detection was developed. The platform can automatically conduct RNA extraction and purification, multiplexed reverse transcription LAMP (RT-LAMP) detection, and real-time fluorescent monitoring of amplicon. LAMP or RT-LAMP has been demonstrated on many types of centrifugal platforms that gives a shorten time consumption facilitating the POCT application [18,22]. It is worth stressing that, this is the first time to present the MembR valve as “passive valve” for liquid flow control on the centrifugal disc. The valves were developed by utilizing different hydrophobic or hydrophilic polycarbonate membranes with superfine pore sizes, which provided step-by-step manipulation of five reagents for RNA extraction on the single lab-on-a-disc. With integration of RNA preparation on the platform, it reduces the need of labor-intensive benchtop work and allows the performance by non-professionals. Furthermore, clinical samples of HPAIVs were utilized to demonstrate the applicability of the centrifugal disc in POCT situations with sample-to-answer capability.

2. Materials and methods

2.1. Platform setup

Microfluidic centrifugal platform consisted of a disposable diagnostic chip for the desired bioanalysis and a chip player integrated all the mechanical and electronic component. The configuration of the player is schematically depicted in Fig. 1 which contains: (1) a motor system with rotational speed feedback to provide centrifugal force for fluid actuation and to rotate the chip during detection for consecutive acquisition of fluorescence signal from each reaction chamber; (2) a temperature control system that comprised a heat-conductive baseplate covered with a heat-conductive lid forming a closed heating cavity, several heating elements integrated in the baseplate and lid, and two PT100 sensors for the feedback of cavity temperature; (3) an optical system that introduced a miniature module for fluorescence detection, which integrated a light emitting diode as light source, a 488 nm-narrowband filter, a lens, a dichroic beam splitter, two reflectors and a photo detector in a 55 × 45 × 10 mm cartridge; (4) a control system PCB for temperature control of heating elements, rotational speed control of rotational axis, intensity regulation of light source, data collection, power management, and wireless communication with user's computer. The disposable chip was mounted on the rotational axis inserted on the center of the baseplate for the implementation of a constant, non-contact heating by hot air.

2.2. Valve testing disc fabrication

The testing disc of 60 mm diameter consisted of, from top layer to bottom layer, a 0.1-mm thick poly (ethylene terephthalate) (PET) film layer with strong self-adhesive (Shanghai Shuliu Technologies Ltd., China), a 2-mm thick top patterned layer, a MembR valve layer, and a 2-mm thick bottom patterned layer. The patterned layers of disc were fabricated of transparent photosensitive resin (Yinmeng 9800, Shanghai Yinmeng Technologies Co., Ltd., China) with stereolithographic print by using a 3D printer (SLA 660, ZRapid Technologies Co., Ltd., China). For valve fabrication, the polycarbonate membranes were coated with a hydrophobic coating reagent (Scotchguard, 3M, USA) for hydrophobic modification and completely dried prior to usage. The valve layer